PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below
International application No. PCT/EP2004/009156	International filing date *(day/month/year)* 16.08.2004
Priority date *(day/month/year)* 29.08.2003	
International Patent Classification (IPC) or both national classification and IPC C12N15/10	
Applicant QIAGEN GMBH	

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/009156

Box No. II Priority

1. ☐ The following document has not been furnished:

   ☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4,5,8,19-23,31,32
	No:	Claims	1-3,6,7,24-30
Inventive step (IS)	Yes:	Claims	1-32
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-32
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1). Present Application.

The present application pertains to :

claim 1 : A method for isolating a biological target material from other material in a medium comprising the steps of:

a) providing a medium including the biological target material;
b) providing magnetic particles capable of reversibly binding the biological target material;
c) forming a complex of the magnetic particles and the biological target material by combining the magnetic particles and the medium in the presence of a salt and a volume excluding agent;
d) removing the complex from the medium by application of an external magnetic field; and
e) separating the biological target material from the complex by eluting the biological target material, whereby the isolated biological target material is obtained.

claim 26 : Use of a method according to any of the forgoing claims for isolating a biological target material from other material in a medium.

claim 29 : A kit to perform a method according to claims 1 to 25, the kit comprising an aliquot of magnetic particles suspended in an aqueous solution in a first container and, optionally, other components needed to perform a method according to claims 1 to 25.

2). Cited Documents.

The following documents are referred to in this communication:

D1 : US 5 705 628
D2 : US 5 898 071
D3 : EP 1 069 131
D4 : WO 02/066993
D5 : EP 0 757 106
D6 : WO 98/31840

D1 discloses (abstract) a method of separating polynucleotides such as DNA and RNA (c.f. cl.2 and 3 of the present application) from a solution by reversibly binding them to a magnetic microparticle, in the presence of a salt and polyalkylene glycol. After the polynucleotides have been bound to the magnetic microparticles to form a complex, they are separated from the solution - e.g. by applying a magnetic field (col.6, l.5), and the polynucleotides eluted (col.6, l.8-12).

The salt is selected from a list (col.5, l.47-52) including LiCl (c.f. cl.6 and 7 of the application), and NaCl (c.f. cl.9 and 10 of the application), and present in a concentration of between 0.5 and 5.0M (col.5, l.56-58 - c.f. cl. 11 of the present application).

The polyalkylene glycols are preferably polyethylene glycol and polypropylene glycol (col.5, l.36-7 - most preferably the former - c.f. cl. 12-14) - preferably present in a concentration of about 7% to about 13% (col.2, l.47 - c.f. cl.18). The molecular weight of the PEG is in the range of 6000-10000, with 8000 being preferred (c.f. cl.15-17).

In one embodiment, microparticles with bound DNA are washed (col.6, l.30-5 - c.f. cl.24)

D1 also refers to a kit containing magnetic particles (col.1, l.56-63 - c.f. cl.29).

Thus the subject matter of claims 1-3,6,7,9-18,24-30 lacks novelty in the sense of Articles 33(1) and 33(2), PCT.

The only differences between the present application and D1 are the use of silica/siliceous oxide coated magnetic particles (not specified in D1 - claims 4,5,31,32); the use of a specific chaotropic salt as defined in claim 8; and the use of a specific

concentration of the volume excluding agent (claims 19-21).

D2 is ostensibly similar to D1.

D3 outlines a similar process to D1 and D2, but uses magnetizable cellulose particles with 10%PEG 8000 as the binding buffer - **D4** is similar to D3.

D5 describes a nucleic acid bondable magnetic carrier which comprises silica magnetic particles. They can be used to isolate nucleic acids by attaching themselves to the molecules to be isolated and applying a magnetic field. The desired molecules are then eluted from the magnetic silica particles. The process also uses a chaotropic agent (list on p.3, l.53-54). However, there is no mention of volume excluding agents.

D6 is similar to D5 and also uses silica magnetic particles - on page 14 it discusses a method for preparing these magnetic substrates by depositing a siliceous oxide coating on magnatite core particles.

3). Inventive Step - Articles 33(1) and (3), PCT.

In so far as it is novel, the present application only differs from that of the closest prior art (D1) in the use of silica/siliceous oxide coated magnetic particles (not specified in D1 - claims 4,5,30,31); the use of a specific chaotropic salt as defined in claim 8; and the use of a specific concentration of the volume excluding agent (claims 19-21).

There is no evidence in the application that any of these three features give rise to unexpected/improved effects. Thus the problem to be solved by the present application is regarded as the provision of a further process for isolating biological target materials.

D5 discloses a method for isolating nucleic acids from biological materials comprising the use of magnetic silica particles, a chaotropic agent (including guanidine salts, sodium iodide, potassium iodide, sodium thiocyanate and sodium isothiocyanate). It does not detail the volume excluding agent. D6 also discloses similar features to D5.

Thus, the person skilled in the art, when wishing to solve the above mentioned problem is clearly provided (in D5 or D6) with an incentive to use magnetic silica particles or the specific chaotropic agents mentioned above. Moreover, since there is no technical

effect associated with the change in concentration of the volume excluding agent, the person skilled in the art would naturally think of using different concentrations with a view to optimising the process for automation.

Thus the subject matter of claims 1-32 cannot be regarded as involving an inventive step in the sense of Article 33(3), EPC.

Re Item VII
Certain defects in the international application

Lack of Disclosure - Article 5, PCT: Lack of Support - Article 6, PCT.

Not only are many of the above terms unclear, but they are speculatively broad - they are neither disclosed in the application as a whole, nor supported by what is present in the application. This is especially the case for :

- biological target material
- magnetic particles
- volume excluding agent.

Re Item VIII
Certain observations on the international application

Clarity - Article 6, PCT.

There are a number of clarity objections listed here :

Claim 1
Claim 1 as a whole appears to lack several essential features which are important for carrying out the claimed invention.

i). the term "biological target material" is unclear.
ii). the term "other material" is unclear.
iii). the word comprising is not limiting, it means that not only could other steps be present, but that those that are present may be carried out in a different order from a).-

e)., whilst not all these combinations would work.
iv). the word "providing" in steps a). and b)., along with "forming", "combining", "removing", "separating" and "eluting" in steps c).-e). are, in effect, results to be achieved, since they detail what the result should be, but not how they are carried out.
v). the wording "magnetic particles capable of reversibly binding the biological target" is a result to be achieved. Similarly the expression "whereby the isolated biological target is obtained".
vi). the term "volume excluding agent" is unclear, and also attempts to define the agent in terms of a result to be achieved.

Claim 7
The term "and/or" is unclear - it could mean that either sodium perchlorate may be selected along with sodium trichloroacetate, or that all the preceding agents could be selected with sodium trichloroacetate. Furthermore, there is no apparent support for the assertion that these groups can be combined.

Claim 12
The term "and/or" is unclear - it could mean that either amylose may be selected along with polyvinyl alcohol, or that all the preceding agents could be selected with polyvinyl alcohol. Furthermore, there is no support for the assertion that these groups can be combined. Similarly for claim 13.

Claim 22
Refers to silica magnetic particle according to claim 1, but claim 1 does not specify that the magnetic particle is silica.

Claim 24
The terms "washing and elution buffers known per se" is unclear.

Claim 27
This claim attempts to define the subject matter for protection in terms of a result to be achieved. Moreover, the word "clustering" is relative and not very clear - how closely can something be associated before it is regarded as clustering ? The term "fluidic medium" is also relative - some gaseous compositions can be regarded as fluidic, whilst it is thought that the applicant wishes this term to mean liquid.